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MANAGEMENT OF HARD TISSUE AVULSIVE WOUNDS AND MANAGEMENT OF ORO--ETC(U)

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REPORT NUMBER 5

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ANNUAL REPORT

Larry G. McCoy, Craig R. Hassler,
and Dale E. Niesz

July, 1976

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20314

Contract No. DADA17-69-C-9118

BATTELLE
Columbus Laboratories
505 King Avenue
Columbus, Ohio 43201

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This report summarizes the results of continued research studies for the further development and understanding of the in vivo behavior of resorbable calcium phosphate ceramics for use in the management of hard tissue avulsive wounds and orofacial fractures.

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Specific studies have been devoted primarily to the preparation and comparative in vivo evaluation of experimental porous tricalcium phosphate ceramics having high resorption rates. Secondary studies included the preparation of experimental tricalcium phosphate materials having controlled pore size distributions for future in vivo resorption rate studies and a determination of the feasibility of fabricating porous calcium orthophosphate as a potential high resorption rate implant material. The results of the in vivo studies suggest that the achievement of high resorption rates may not be desirable particularly in stress-bearing situations.

Recommendations for future materials development and implant studies are presented.

FOREWARD

This study has been conducted at Battelle's Columbus Laboratories utilizing the talents and resources of the Ceramic Materials Section and the Bioengineering/Health Sciences Section. This is the Fifth Annual Progress Report under Contract No. DADA17-69-C-9118, "Management of Hard Tissue Avulsive Wounds and Management of Orofacial Fractures". The Principal Investigator for this research was Mr. Larry G. McCoy. The Principal Physiologist for the animal implant studies was Dr. Craig Hassler.

We would like to acknowledge the valuable assistance of Mr. Roger K. Beal for his excellent work in preparation of the porous implant materials, Mr. Lynn C. Clark for the excellent histologic preparations, and Mrs. Joan H. Rotaru for the expert radioisotope analyses.

In conducting the research described in this report, the investigators have adhered to the "Guide for Laboratory Animals Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council.

The data on which this report is based may be found in Battelle Laboratory Record Books 29722, 30217, and 30409.

ABSTRACT

This report summarizes the results of continued research studies for the further development and understanding of the in vivo behavior of resorbable calcium phosphate ceramics for use in the management of hard tissue avulsive wounds and orofacial fractures.

Specific studies have been devoted primarily to the preparation and comparative in vivo evaluation of experimental porous tricalcium phosphate ceramics having high resorption rates. Secondary studies included the preparation of experimental tricalcium phosphate materials having controlled pore size distributions for future in vivo resorption rate studies and a determination of the feasibility of fabricating porous calcium orthophosphate as a potential high resorption rate implant material. The results of the in vivo studies suggest that the achievement of high resorption rates may not be desirable particularly in stress-bearing situations.

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MANAGEMENT OF HARD TISSUE AVULSIVE WOUNDS
AND MANAGEMENT OF OROFACIAL FRACTURES

by

Larry G. McCoy, Craig R. Hassler,
and Dale E. Niesz

SUMMARY

Research studies were continued to further the development and understanding of the in vivo behavior of resorbable calcium phosphate ceramics for use in the management of hard tissue avulsive wounds and orofacial fractures.

Material processing and fabrication studies were conducted to develop porous tricalcium phosphate materials having higher resorption rates. By the utilization of precise formulation and batching procedures and careful processing procedures, significant improvements in the structure and chemical composition of porous implant materials has been achieved.

The results of in vivo evaluations conducted in the rabbit calvaria using this improved material indicate that significant improvements in the resorption rate, in comparison to an historical standard material, have been achieved. In fact, the improved material degraded so rapidly after the ninth month that connective tissue invades the implant. This result does not imply lack of biocompatibility but does suggest that such rapid degradation could be deleterious in stress-bearing situations. It is not known whether the enhanced resorptivity results from achieving a Ca/P ratio closer to the theoretical for tricalcium phosphate or from the improved structural uniformity. Future studies should be directed toward determining the relative importance of these two factors.

Further material processing and fabrication studies were conducted to develop experimental porous tricalcium phosphate implant materials having controlled pore size distributions for in vivo evaluation of the effects of structural variations or resorption rate. Significant

fabrication difficulties were encountered in terms of reproducibly achieving the desired pore structures. A partial solution was provided by utilization of a technical grade naphthalene in place of the reagent grade naphthalene normally used as the fugitive pore forming agent. The adoption of thorough and careful phosphate/naphthalene powder blending techniques was found necessary to achieve the structural uniformity and reproducibility desired.

Additional material fabrication studies were conducted to determine the feasibility of developing porous calcium orthophosphate, $CaHPO_4$, as a new, potentially higher bioresorbable implant material. Fine-grained specimens up to 70 percent dense were successfully prepared by reactive hot-pressing techniques. However, the preparation of specimens containing coarse porosity has not been possible and future success does not appear likely using this technique.

BACKGROUND, PROBLEM, AND APPROACH

Historically, man has employed various techniques in the repair and/or treatment of osseous diseases, deformities, and wounds. A brief review by Hulbert, et al.[1] on man's attempts to repair bone defects reveals that early studies were conducted using both treated and untreated heterogeneous, homogeneous, and autogenous bone for bone grafting applications. Of these, autogenous bone grafting remains the most satisfactory approach but is not without disadvantages. Autogenous bone grafting involves transplantation of healthy bone from one area to a defect area, thus requiring a double operation. Also, the procedures are not always conducive to repair of massive osseous defects.

Porous ceramics have been developed in many laboratories and experimentally evaluated as permanent prostheses for partial replacement of hard tissue[1,2,3]. Also, some material characteristics related to biocompatibility and structural design have been defined[4]. Some ceramics have potentially desirable properties for prosthesis application. These

properties include a chemical inertness or lack of reactivity with body fluids and a propensity toward permitting hard and soft tissue ingrowth[3,5-7]. However, most ceramics are inherently brittle and subject to easy fracture. Nonresorbable porous ceramics, once proliferated by bone, tissue, and blood vessels, in theory become an integral and permanent part of the repaired member section.

Of particular interest to the current study using resorbable ceramics are the historical results of using plaster of paris as a filler material in bone defects[5]. As early as 1892, plaster of paris was used to facilitate repair of bone diseases and defects. Since those early studies, other investigators have experimented with plaster of paris in orthopedic surgery. In general, results reported were encouraging and, in general, showed some indications of success. Little information is available on the mechanism or sequence of events of plaster of paris resorption and the possible adverse biological effects. However, the studies make apparent the feasibility of using a temporary, bioresorbable material to facilitate the repair of bone defects.

Since April, 1970, Battelle's Columbus Laboratories has been conducting research under contract with the Dental Research Division, U. S. Army Medical Research and Development Command, on the development of resorbable ceramics for potential application in the repair of hard tissue avulsive wounds. The basic materials have been calcium phosphates. These materials were selected because they contain two of the essential elements of the nature bone mineral phase, calcium hydroxyapatite. Initially, porous calcium phosphates consisting of the mineral phases $Ca(PO_3)_2$ or $Ca_3(PO_4)_2$ were developed. Low-density porous materials were selected for development as it had been shown that bone could proliferate into porous ceramics[3,8].

In vivo studies were conducted at USAIDR using the sintered porous materials and slurries prepared from tricalcium phosphate $Ca_3(PO_4)_2$ and other calcium orthophosphate powders $CaHPO_4$ and $Ca(H_2PO_4)_2$ to evaluate the potential use of calcium phosphates to both facilitate repair of bone

defects and to determine the best material for further exploration[9,10]. The implant studies have indicated that calcium phosphates consisting essentially of the mineral phases $Ca(PO_3)_2$, $Ca_3(PO_4)_2$, and $CaHPO_4$ are well tolerated by the tissue, appear to be nontoxic, are resorbable, and permit rapid invasion of new bone.

Of the various porous calcium phosphate materials investigated, tricalcium phosphate, $Ca_3(PO_4)_2$, was selected for continued development and evaluation since it was easy to fabricate and was found to be both biocompatible and resorbable.

In early work, the emphasis was directed toward producing low-density porous materials consisting of a single phase tricalcium phosphate for biocompatibility implant studies. Continued research efforts were directed toward the development of higher density materials with particular emphasis on improving strength. For cases involving extensive bond loss, slower adsorption rates were desired. This would permit the ceramic to
slower adsorption rates were desired. This would permit the ceramic to
become a part of the temporary mechanical fixation and permit earlier function of the repairing member.

Previous improvements in preparation and fabrication procedures provided block materials having strength values of approximately 4000 psi. However, the brittle fracture characteristics of this high-density material have precluded its successful application when standard screw and plate fixation techniques are employed. Since the strength of this material was considered below workable values for sectional bone replacement, efforts continued toward improving the strength of block materials by refinements in powder preparation and fabrication procedures. Average diametral tensile strengths of 7400 psi have been achieved in 88 percent dense materials. This represents a 3- to 4-fold increase over the strength of some of the earlier materials. Although no direct measurements have been made, the strengths of porous block implant materials should be proportionally improved. Efforts to provide further strength improvements are continually in progress.

Although previous implant studies at USAIDR have demonstrated that porous tricalcium phosphate is biocompatible, resorbable, and promotes or permits rapid ingrowth of new bone, recent histological evidence indicates

persistance of a residual ceramic structure as long as 1 year after implantation. This structure appears to be composed of an isomorphic distribution of small encapsulated ceramic particles. The presence of this residue would be expected to retard complete remodeling of the bone and the attendant strength development.

As a result of this problem, primary emphasis has been shifted back to the development of porous materials having improved (increased) resorption rates. This objective may be achieved by changes in structure or chemistry of the ceramic implant material.

To provide additional information on the in vivo behavior of the tricalcium phosphate bioresorbable ceramics, implant studies were initiated at BCL using the rabbit calvarium model. Historic samples of tricalcium were implanted as a control and samples of two new materials were implanted for comparative observation. These new materials were prepared using the improved processing techniques derived in previous materials development studies and represent significant improvements in the structural characteristics of porous tricalcium phosphate. One of the new materials was radiolabeled with Ca^{45} isotope to assess the fate of calcium from the implant in the total body pool. The characteristics of the materials involved and the results of these in vivo studies are the major subject of this report.

The preliminary results of these in vivo studies (i.e., the radiological evidence) indicating improved resorptivity with improved pore structure have become the basis for continuing porous material development studies and proposed in vivo studies. This effort has involved the preparation of experimental porous tricalcium phosphate materials of a fixed composition having their pore-size distributions modified with the intent of improving resorption rates. Studies have also been conducted to determine the feasibility of developing consolidated porous forms of calcium orthophosphate, $CaHPO_4$, as a new bioresorbable implant material.

MATERIALS AND METHODS

Porous Materials for Current In Vivo Studies

To assess the ultimate fate of resorbable tricalcium phosphate ceramics in the body tissue and fluids, the relative dissolution rates, and the relative ingrowth rates, large segments of three porous materials were prepared for placement into rabbit calvaria. These materials included: (1) A historic sample of porous tricalcium phosphate as the experimental control, (2) a radiolabeled porous tricalcium phosphate, and (3) a chemically or physically modified material intended to have a higher resorption rate.

Radiolabeled Material

To determine the appropriate dosage of Ca^{45} isotope required in the radiolabeled implants, preliminary studies were conducted to develop a model for calcium metabolism in adult rabbits. The derived model is shown in Figure 1 in a compartmented form adapted from Dolphin and Eve[11]. Based on the model and the methodology requirements (i.e., the required counts/minute, counting efficiency, dilution factors, etc.) it was determined that an isotope concentration or specific activity of 100 microcuries/gram of implant material would be adequate to provide reasonable measurement accuracy (1 percent) within approximately 10 minutes scintillation counting time on an assumed daily urine sample of 50 ml. On this basis each implant ($\sim$500 mg) had a specific activity of 50 microcuries.

In anticipation of the preparation of the radiolabeled implant material, extensive preliminary efforts were directed toward the development of scaled-down laboratory procedures for the preparation of small batch quantities (20 grams) of tricalcium phosphate powder and the fabrication of small block specimens of the porous material. The objective of these studies was to produce material having chemical and physical properties comparable to the historical control specimens. However, as a result of the utilization of improved powder preparation procedures developed in earlier processing studies (i.e., batching, calcination, and ball milling)

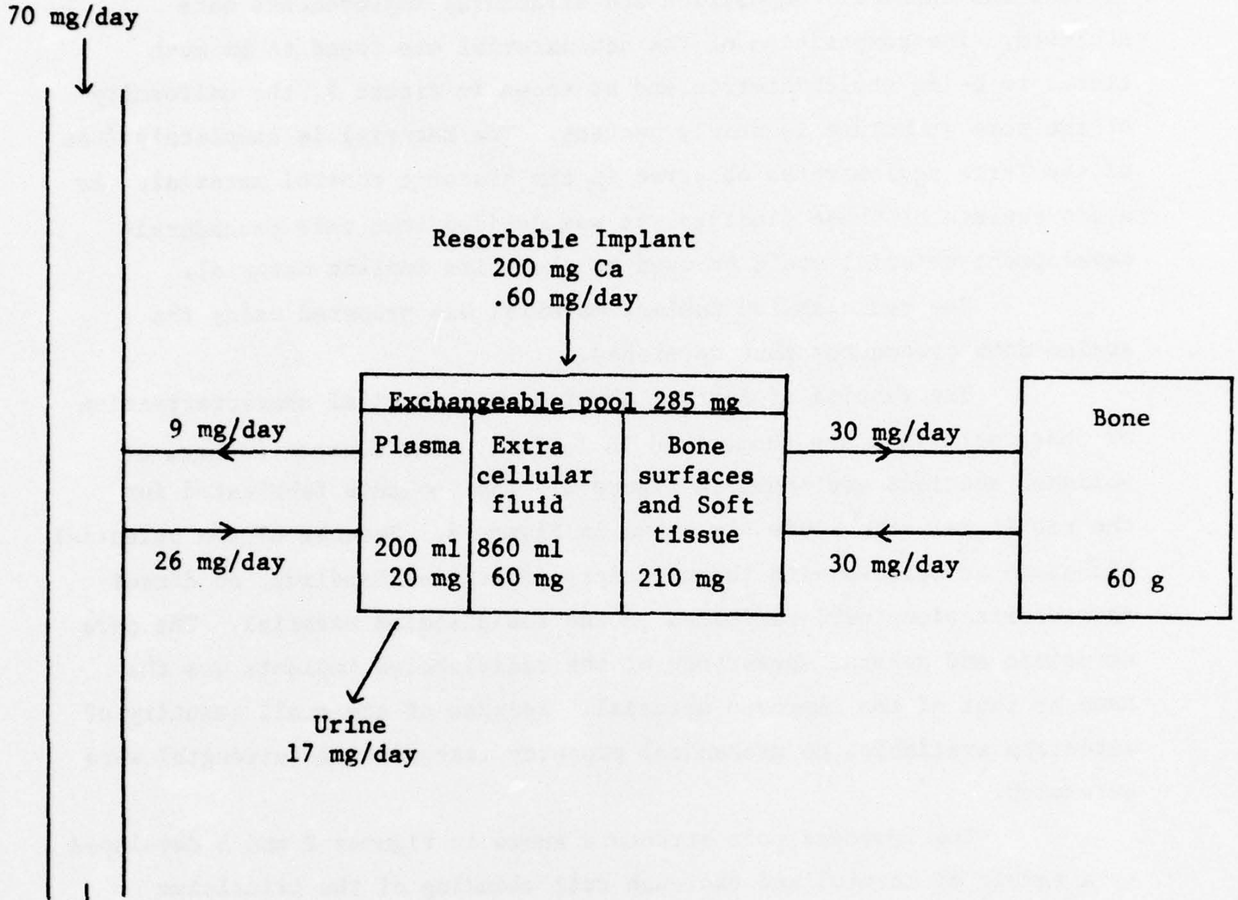


FIGURE 1. COMPARTMENTED MODEL OF CALCIUM METABOLISM IN ADULT RABBITS

significant chemical composition and structural improvements were achieved. The composition of the new material was found to be much closer to being stoichiometric and as shown in Figure 2, the uniformity of the pore structure is nearly perfect. The material is completely free of the large agglomerates observed in the historic control material. As a consequence of these findings, it was decided that this procedural-development material would be used as the third implant material.

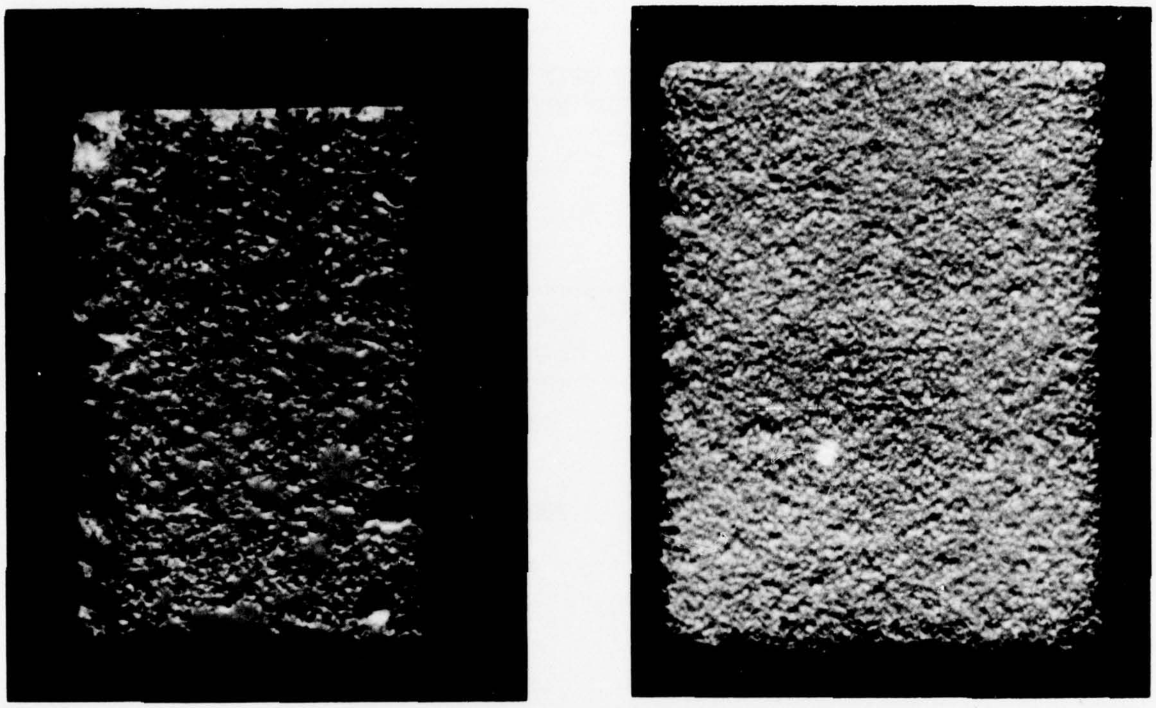
The radiolabeled implant material was prepared using the scaled-down procedures thus developed.

The results of further chemical and physical characterization of these materials are summarized in Table 1. The microstructures of polished sections are shown in Figure 3. The implants fabricated for the rabbit calvaria study are shown in Figure 4. Because of the potential biohazard associated with the necessary additional handling, no direct characterizations were performed on the radiolabeled material. The pore structure and general appearance of the radiolabeled implants was the same as that of the improved material. Because of the small quantity of materials available, no mechanical property measurements (strength) were attempted.

The improved pore structure shown in Figures 2 and 3 developed as a result of careful and thorough roll blending of the tricalcium phosphate powder with the naphthalene pore forming agent. Before pressing and sintering, the blended powders were screened through a 20-mesh sieve to remove or break-up powder agglomerates which can form the large dense regions observed in the historical control material.

The improvement in the chemical composition (Ca/P ratio) of the new materials did not result as a conscious effort to simultaneously change both compositional and structural parameters of the material. Rather, this improvement occurred as a result of the application of improved formulation and batching procedures developed in earlier studies.

For the preparation of tricalcium phosphate powder, tribasic calcium phosphate, $Ca_{10}(OH)_2(PO_4)_6$, is hydrothermally reacted with phosphoric acid to adjust the phosphate content. Historically, the



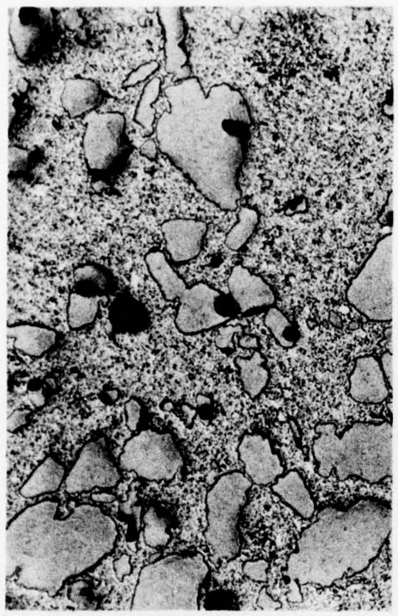
4X
Historical Control Material

4X
Improved Material

FIGURE 2. A COMPARISON OF THE STRUCTURAL CHARACTERISTICS OF THE HISTORICAL CONTROL IMPLANT MATERIAL AND THE IMPROVED TRICALCIUM PHOSPHATE MATERIAL

TABLE 1. PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE TRICALCIUM PHOSPHATE IMPLANT MATERIALS

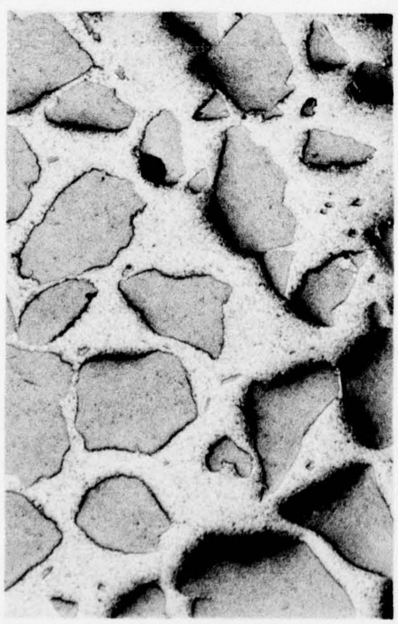
	Historical Standard	Improved Material	Radiolabeled Material	Theoretical
Density (percent theoretical)	47.7	43.0	41.0%	--
Average Pore Size (microns)	180	208	N.D.	282
Chemical Analysis (weight percent)				
Ca	36.1	37.3	N.D.	38.8
P	20.6	20.1		20.0
O	43.5	42.6		41.3
Ca/P	1.75	1.86		1.94



150X

250X

Historical Standard



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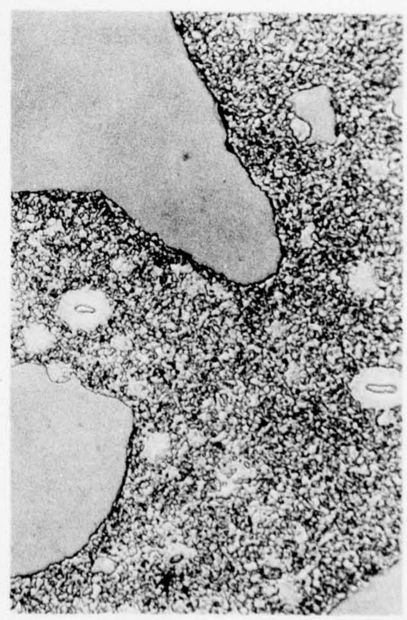


250X

Improved Material

FIGURE 3. MICROSTRUCTURES OF THE POROUS TRICALCIUM PHOSPHATE IMPLANT SPECIMENS (Polished Sections)

FIGURE 4. IMPLANTS PREPARED FOR THE RABBIT CALVARIA STUDY FROM STRUCTURALLY IMPROVED MATERIAL

theoretical analyses of these reagents were used to formulate the batches, and excess phospheric acid was added to compensate for the assumed vaporization losses during calcination and sintering. The resulting materials were subsequently found to be phosphate rich, and marked differential grain growth attributed to liquid phase sintering was observed in the microstructures. The principal compositional improvements (Table 1) were achieved by formulation on the basis of the actual (label) analyses of the reagents and elimination of the excess phospheric acid addition. Further fine tuning of the composition can be achieved by iterative batching and analysis procedures.

Porous Tricalcium Phosphate Processing Studies

On the basis of the encouraging preliminary in vivo results achieved with the improved pore structure implant materials described above (i.e., significantly enhanced resorption as evidenced by 3- and 6-month radiographs), continued material processing studies were directed toward the preparation of structurally modified porous materials for future in vivo studies. The objective of these studies was to determine if the pore structure of a standard or fixed composition material could be selectively modified to further enhance the resorption rate.

The first task in this study was to produce a new "standard" material which duplicated the pore structures of the improved materials described above. The achievement of this objective was not as simple as originally anticipated. Initial efforts to scale up the phosphate/naphthalene powder blending process to accommodate 50-gram quantities of the milled tricalcium phosphate powder were not successful. Stable distributions of the naphthalene pore forming agent could not be achieved, thus resulting in the development of nonuniform pore distributions and agglomerated structures in the sintered ceramic.

Extensive blending studies were then initiated to determine the conditions required for achieving optimum blends using 10-gram quantities of phosphate powder as used in the preliminary isotope processing studies. Because of the lack of adequate techniques to qualitatively or

quantitatively define the character of the phosphate/naphthalene blends, an iterative blending-sintering-structural examination procedure was utilized to assess the parametric relationship between material, process, and product characteristics. Prior to pressing and sintering, the blends were examined microscopically to determine what characteristics contributed to good or poor structural development in the sintered specimen. Narrative descriptions of the blends and photographs of back-lighted thin-sections of the sintered specimens (See Figure 5) were maintained for a comparative record. Only in extreme cases could segregation of the powder blends be observed which attributed to inhomogeneous structures in the sintered specimens.

After several blending, pressing, and sintering iterations, it became apparent that uncontrolled factors related to the characteristics of the reagent-grade naphthalene pore-forming agent were seriously influencing the results. The blending process appears sensitive to variations between lots of reagent naphthalene, the freshness of the naphthalene, and to the temperature and humidity conditions in the laboratory at the time of preparations. Trial fabrications indicate that the process is relatively insensitive to the condition of the phosphate powder (i.e., calcined condition, milled condition, and dryness). Also, uncontrolled variations in the particle size distributions of the -40/+100 mesh fraction and of the particle shape of the reagent-grade naphthalene are sufficient to produce significant structural variations in the sintered block specimens from which the rabbit calvarium implants are cut. Rectangular block shapes are made to minimize material cutting losses. Since the phosphate powder/naphthalene blend is first die pressed to shape before final hydropressing, a preferential orientation of the plate-like reagent naphthalene particles parallel to the punch faces of the die has been observed.

Because of the difficulties encountered in obtaining optimum and consistent blends and the above structural inhomogeneities associated with the use of reagent grade naphthalene, processing studies were initiated using a technical grade of naphthalene*. This substitution was made

* Standard Naphthalene Products Co., Inc., Kearny, New Jersey, 07032, USDA Reg. No. 1814-4.

on the supposition that the refinement process used for the preparation of the reagent grade naphthalene removes light hydrocarbon "impurities" which may be beneficial to the development of stable, dispersed powder blends. Also, the refinement process results in the formation of coarse flake particle shapes which can result in preferentially oriented pore structures.

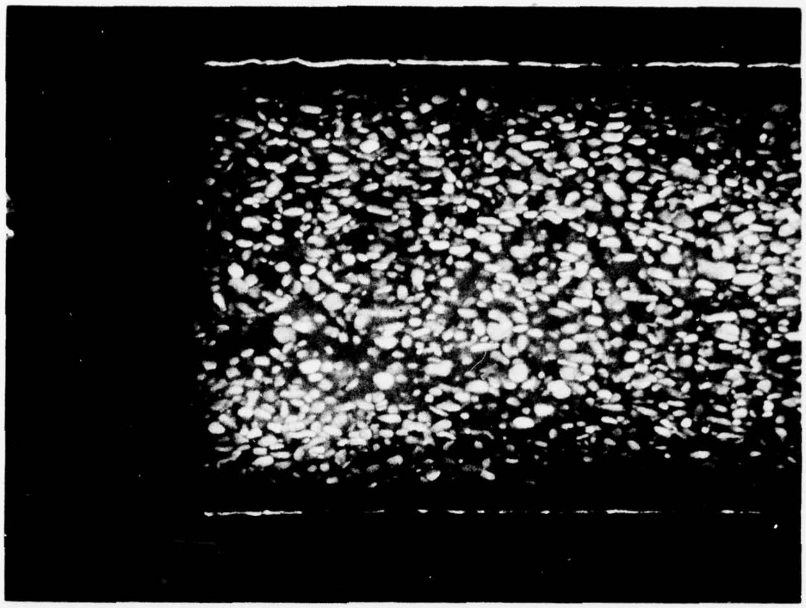
Shown in Figure 5 are the pore structures of two tricalcium phosphate blocks prepared using each type of naphthalene. A comparison of the structures indicates that the use of the technical grade naphthalene does, in fact, produce more uniform and less oriented structures. Subsequent laboratory experience indicates that satisfactory blends can be achieved easier and more consistently using the technical grade material.

Before adopting its use for the preparation of implants, a check was conducted to determine the ash content of the technical grade material. Approximately 5 gram samples of both technical and reagent naphthalene were vacuum outgassed at 250 F for 16 hours. Both left a slight resinous or waxy deposit on the watch glass. After subsequent outgassing at 500 F there was no observable or measurable residue. Based on these results, the use of technical grade naphthalene has been adopted for preparation of subsequent implant specimens.

Laboratory work has been completed on the fabrication of experimental porous implant specimens having pore size distributions modified for improved resorption rates. Three materials are being prepared:

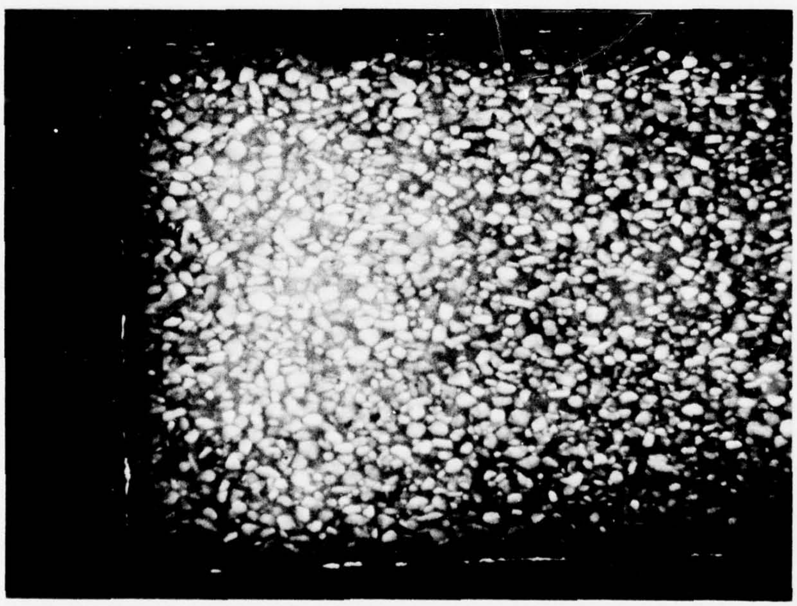
(1) A standard material having a pore size distribution corresponding to the natural particle size distribution of naphthalene between 150 and 420 microns (-40/+100 mesh).

(2) A material modified to increase the percentage of porosity in the 150 to 250-micron range prepared by using a modified naphthalene particle size distribution



7X Specimen D22-E20

(a) Prepared With Reagent Grade Naphthalene. Note the Inhomogeneity and Preferential Horizontal Orientation.



7X Specimen D22-E18

(b) Prepared With Technical Grade Naphthalene. Note the Uniformity and Lack of Preferential Orientation.

FIGURE 5. A COMPARISON OF THE PORE STRUCTURE OF SINTERED BLOCKS OF TRICALCIUM PHOSPHATE PREPARED WITH (a) REAGENT GRADE NAPHTHALENE AND (b) TECHNICAL GRADE NAPHTHALENE (Back-lighted thin sections, the light areas are pores and grey areas are wall sections or agglomerated regions between pores)

(3) The standard material having increased microporosity in the 35-45-micron range induced by the addition of a small amount of 40 micron spectrographic-grade graphite powder.

The characteristics of these materials are summarized in Table 2 and their microstructures are shown in Figure 6. Initially a 10 percent microporosity material fabricated first was found to be too weak and friable for cutting and shaping of implants and was, therefore, remade using only 5 percent graphite additive. However, the 5 percent material sintered to nearly the same density as the standard. As a compromise, a composition containing 7.5 percent graphite additive was fabricated as the microporosity implant material.

No surgical implant studies of these materials have been initiated pending completion of the in vivo studies discussed in a later section of this report. To reiterate, the preliminary radiography evidence indicated that modifications of pore structure to increase resorption rate were a desirable objective. The fine graphite pore forming agent was added to the third material to enhance its resorption rate by increasing the permeability and internal surface area of the wall between the large pores. However, based on the most recent histological evidence, this approach may not be as desirable as originally supposed. Further review of the in vivo results is needed before proceeding with implant studies on this materials.

Calcium Orthophosphate Processing Studies

Documentation exists in the literature on in vitro and in vivo calcification processes which indicates both $CaHPO_4$ (calcium orthophosphate) and $Ca_3(PO_4)_2$ (tricalcium phosphate) may act directly as precursor materials for precipitation and epitaxial growth of hydroxyapatite[12,13] or indirectly by the formation of intermediate crystalline or amphorous calcium phosphates[14,15,16]. Also, a recent study of the mechanisms of hydrolysis of calcium phosphates[17] indicates that an equilibrium boundary exists

TABLE 2. MODIFIED PORE STRUCTURE EXPERIMENTAL IMPLANT MATERIALS

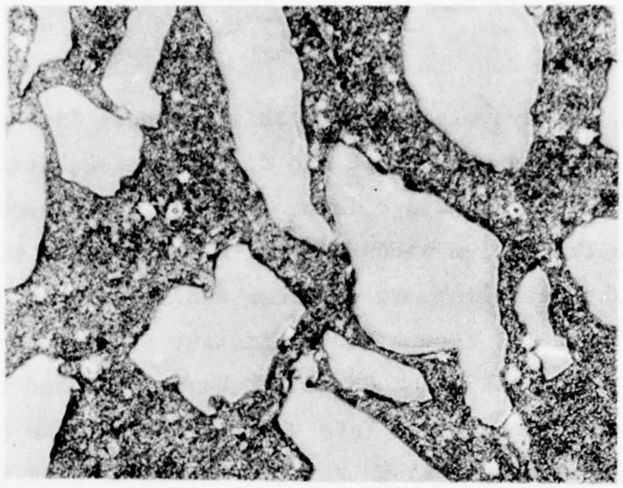
Batch[(a)] Number	Designation	Naphthalene[(b)] Pore Size Distribution	Additive[(c)] Weight Percent	Sintered Density Percent
E22	Standard	A	None	48.6
E26	Modified-Fine	B	None	47.0
E35	Micro 7.5	A	7.5	46.5

(a) All specimens were prepared using Batch D-22 tricalcium phosphate powder calcined at 1500 F and ball milled for 20 hours in hexane. All specimens were sintered 4 hours at 2050 F.

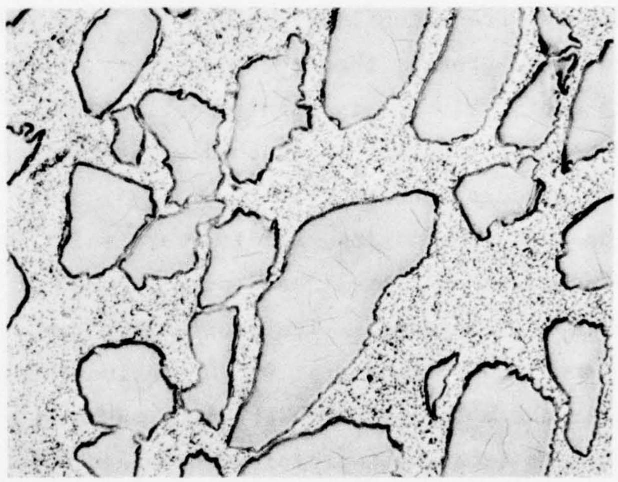
(b) Naphthalene particle size distributions used (weight percent);

	Mesh Sizes		
	-40/+60	-60/+80	-80/+100
A	76	18	6
B	40	20	40

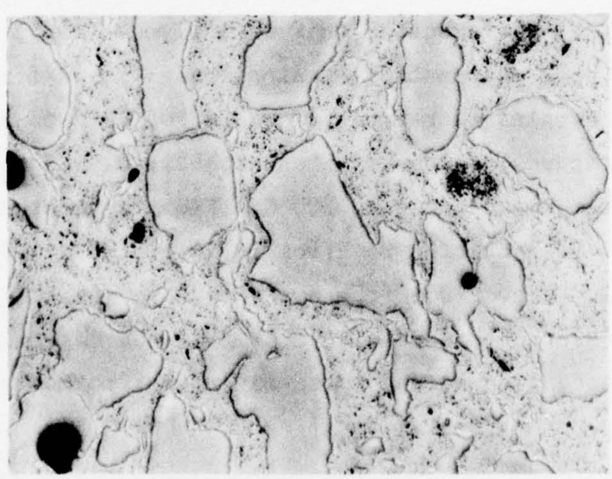
(c) The additive used to induce microporosity was -325 mesh spectrographic grade graphite.



100X Material E22 8H591



100X Material E26 8H592



100X Material E35 8H593

FIGURE 6. MICROSTRUCTURES OF MODIFIED POROSITY TRICALCIUM PHOSPHATE EXPERIMENTAL IMPLANT MATERIALS

between $Ca_3(PO_4)_2$ and hydroxyapatite. This boundary lies within the ranges of normal blood or plasma pH and dissolved concentrations of both inorganic phosphorus (Σ[P]) and calcium ([Ca^{+2}]). The lack of complete resorption of the tricalcium phosphate implant materials may possibly be explained by the establishment of these equilibrium conditions in vivo.

The results of recent slurry implant studies conducted at USAIDR[10] using both $CaHPO_4$ and $Ca_3(PO_4)_2$ have indicated that the $CaHPO_4$ has a slightly higher resorption rate than the tricalcium phosphate. These resorption rate differences may be related to differences in the particle size of the original powders; however, the solubility properties of these calcium phosphates is thought to contribute significantly to the observed effects. For instance, the solubility product (K_{sp}) of $CaHPO_4$ is approximately five times greater than the solubility of tricalcium phosphate, and its equilibrium pH is significantly lower[12]. Thus, as an alternate higher rate or completely resorbable material, the development of $CaHPO_4$ materials appears attractive.

Based on this information, studies were initiated to determine the feasibility of developing consolidated porous forms of $CaHPO_4$ as a new bioresorbable implant material. The fact that the orthophosphate is a hydrated compound which decomposes at 400 C precludes using standard techniques to sinter the material. The approach taken was to use reactive hot-pressing techniques (i.e., hot-pressing coincident with decomposition reactions) using dibasic calcium orthophosphate ($CaHPO_4 \cdot 2H_2O$) as the starting material.

Initially, differential thermal analyses (DTA), thermogravimetric analyses (TGA), static calcination studies, and X-ray diffraction analyses were conducted on samples of reagent-grade $CaHPO_4 \cdot 2H_2O$ to determine the dehydration temperatures, ranges of phase stability, and the crystalline phases formed at temperatures up to 800 C. From these studies it was determined that the dehydration reaction is initiated at ∿90 C and is complete at ∿300 C. Above 400 C the pyrophosphate ($Ca_2P_2O_9$) is formed.

After several hot-pressing trials it was determined that reproducible 70 percent dense specimens having sufficient strength for

handling and cutting could be formed by heating the dihydrate powder slowly to 360 C while under a continuous load (8,000 to 12,000 psi). X-ray diffraction analysis of the specimens thus formed indicated that the material was single phase $CaHPO_4$. When samples of these specimens were reheated to 600 C, the measured weight loss (7.6 percent) corresponded favorably to the theoretical weight loss for the dehydration of $CaHPO_4$ (6.6 percent). No microstructural or other characterizations were conducted.

However, to have utility as an implant material, specimens having coarse porosity are needed. The only pore-forming agents that have a melting point above the hot-pressing temperature and which can be removed without heating the specimen above the 400 C decomposition temperature of $CaHPO_4$ are water soluble salts. In an attempt to form porous implant specimens, trial hot pressings were conducted using sodium chloride (NaCl) as the pore forming agent. During the hot-pressing cycle the dihydrate should lose approximately 20 percent of its weight as the water of hydration is driven off. With the NaCl present, weight losses approaching 25 percent were measured. This corresponds to the complete decomposition to form pyrophosphate ($Ca_2P_2O_7$) which is confirmed by X-ray diffraction analysis. Subsequent leaching in room temperature distilled water removed all of the salt but also resulted in the formation or appearance of small amounts of calcium chloride and sodium orthophosphate. These results suggest that the presence of the sodium chloride (probably the chlorine) acts to catalyze the decomposition reaction at temperatures where $CaHPO_4$ can otherwise be satisfactorily formed.

Tests conducted to determine the suitability of using graphite as a pore-forming agent in this situation proved unsatisfactory. No measurable weight loss could be achieved by oxidation of a green-pressed powder compact containing 10 percent graphite in flowing pure oxygen after 16 hours at 370 C. Similar tests conducted in an ozone atmosphere provided essentially the same results.

Based on these results it appears that the successful formation of porous $CaHPO_4$ by hot-pressing techniques is unlikely. However, to evaluate the relative resorption rate of a consolidated orthophosphate

material, pellet implant studies could be conducted using the 70 percent dense hot-pressed orthophosphate specimens in comparison to 70 percent dense sintered tricalcium phosphate. Specimens have been prepared but no implant studies are planned at this time.

EXPERIMENTAL ANIMAL STUDIES

This portion of the report will outline the various research procedures which are used in our laboratories to evaluate biodegradable materials. The evaluative procedures include histology, radiography, blood and urine chemistries, radioisotope uptake, and radioisotope distribution studies. The more classical techniques of histology and radiography are the key diagnostic procedures. However, the additional techniques add to our knowledge of the material and its ultimate fate in the animal. The results of our most current research on tricalcium phosphate will be included.

Research Protocol

In order to test the biodegradation of large tricalcium phosphate segments, an experimental model has been devised in this laboratory. We utilize the calvarium of the male, mature, New Zealand white rabbit with a minimum weight of 8 pounds. The calvarium has been found to be an excellent implant site for this relative weak structural biomaterial since stresses upon the calvarium are not extraordinarily high and external stabilization is usually not required. Consequently, confusing effects which might be due to fixation devices are not seen. Of greater importance is the fact that this implant site provides the researcher with a large, relatively uniform area of material for various simultaneous studies. Additionally, periodic radiography of this flat area is an easy matter.

Standard aseptic surgical technique is used to expose the calvarium of the animal. A rectangular portion of the calvarium is osteotomized from the animal. The dimensions of this rectangular patch removed are 0.25 x 0.75 inch. No attempt is made to salvage the periosteum overlying the removed area. The specially shaped portions of tricalcium

phosphate are designed to fit within the above-mentioned rectangular area. The implants are 0.1 inch thick and specially curved to match the curvature of the rabbit calvarium.

Three different experimental groups of animals were followed. The first group consisted of animals implanted with material termed "historical standard tricalcium phosphate". This material is phosphate-rich (Ca/P 1.75) compared to the improved materials now being produced. Additionally, the older formulation has less consistent porosity. The second group of animals were implanted with the materials termed improved formulation tricalcium phosphate which has a more uniform porosity, greater strength, and phosphate ratio closer to natural tricalcium phosphate (Ca/PO_4 1.86). In the third group of animals, the material was the same as the second except radiolabeled Ca^{45} was partially substituted for calcium. Four research animals were included in each group. One animal of each group was sacrificed at intervals of 3, 6, 9, and 12 months.

Blood chemistries of all animals for calcium and phosphorous, as well as urine chemistries for calcium and phosphorous, were taken pre-implant and then for several weeks following implant. Blood and urine chemistries were then followed at 3-month intervals up until the time of necropsy. The animals were radiographed at 3-month intervals until the time of necropsy and then the excised skulls were radiographed postnecropsy. Radiolabeled calcium and tritiated proline were injected 24 hours prior to necropsy. Four hundred μCi of tritiated proline and 200 μCi Ca^{45} were utilized except in those animals which were implanted with radioactive calcium implants. The uptake of the radiolabeled bone metabolism indicators was used as additional indicators of the metabolism occurring within the implant materials. In the radioisotope-implanted animals, the total fate of the calcium from the implant was followed by investigating all major organ systems in the body for radioisotope levels. The histologic analysis consisted of embedding a portion of the excised calvarium and tricalcium phosphate complex in methyl methacrylate and sectioning. Basic

fuchsin stain was utilized. An alternative procedure used was tetracycling labeling of the animals prenecropsy for subsequent analysis by ultraviolet microscopy.

Radiographic Examination of Tricalcium Phosphate Biodegradability

To investigate biodegradability rate noninvasively, research animals were radiographed at 3-month intervals. High-resolution radiographs were obtained using fine-grained industrial film. A Picker industrial X-ray unit was utilized for exposure.

Animal C will be illustrated in this report as an example of the historic control material. Figure 7 shows the material at 3 months postimplant. Note the outline of the implanted material can readily be observed. Figure 8 illustrates the same animal at 6 months postimplant. Note that radiographically the appearance of the implant is not greatly changed; however, a slight decrease in overall radiodensity of the implant can be seen. Figure 9 is the same implant photographed 9 months postimplant. Again, the change in radiodensity is not impressive. Figure 10 is a postnecropsy radiograph of the same animal with the calvarium exposed. The increased resolution with the excised calvarium reveals blotchy areas which are indicative of the nonuniform structure and perhaps selective biodegradation in the material. These radiographs are typical of those seen with the older formulation materials. However, as histology will later indicate, these data alone are insufficient to quantify biodegradability.

This picture is remarkably different when the improved materials are examined. For example, Figure 11 illustrates radiographs taken of Animal K 3 months postimplant. Note that the radiodensity of the implant at this time is already considerably less than that seen at 3 months in the historic standard, Animal C (Figure 7). Figure 12 shows Animal K 6 months postimplant. Note the marked radiolucency about the border of the implant whereas the radiodensity within the implant makes it difficult to

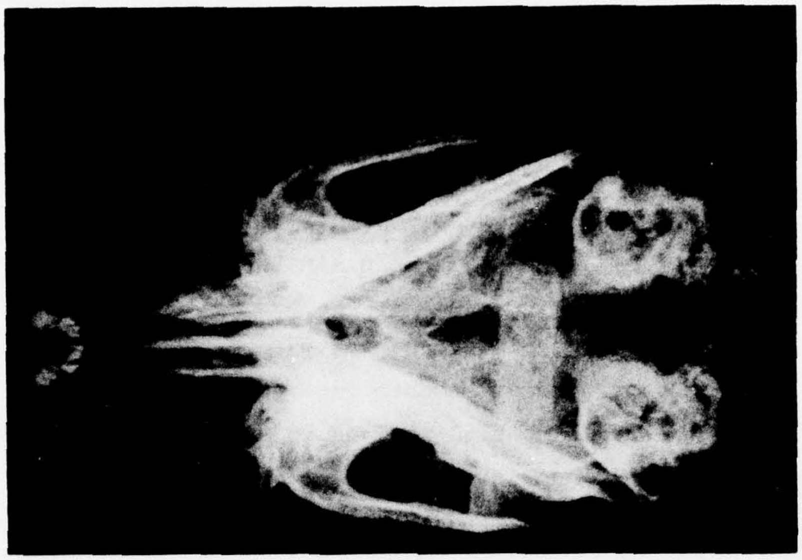


FIGURE 7. RADIOGRAPH OF ANIMAL C 3 MONTHS POSTIMPLANT (HISTORIC STANDARD MATERIAL)

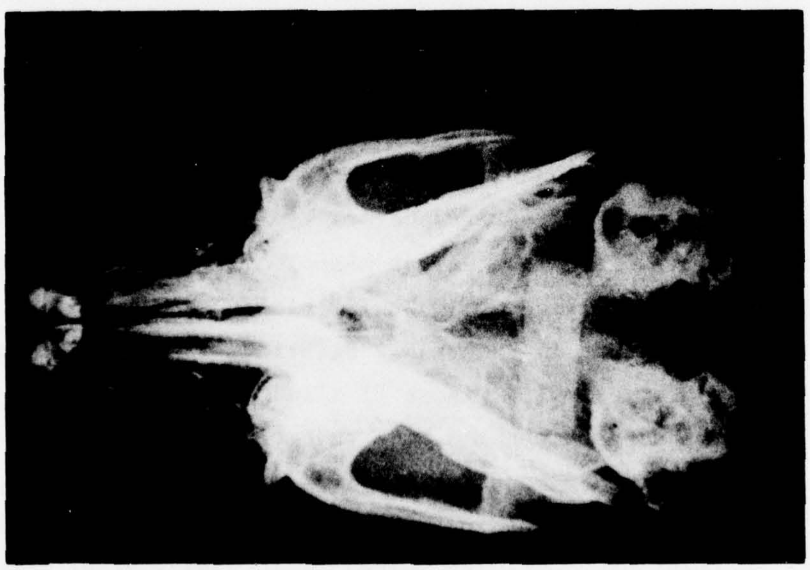


FIGURE 8. RADIOGRAPH OF ANIMAL C 6 MONTHS POSTIMPLANT (HISTORIC STANDARD MATERIAL)

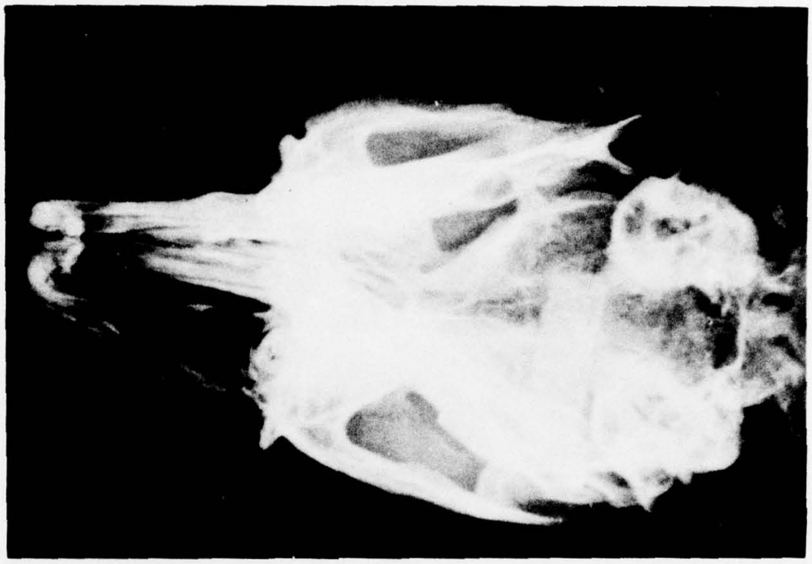


FIGURE 9 . RADIOGRAPH OF ANIMAL C 9 MONTHS POSTIMPLANT (HISTORIC STANDARD MATERIAL)

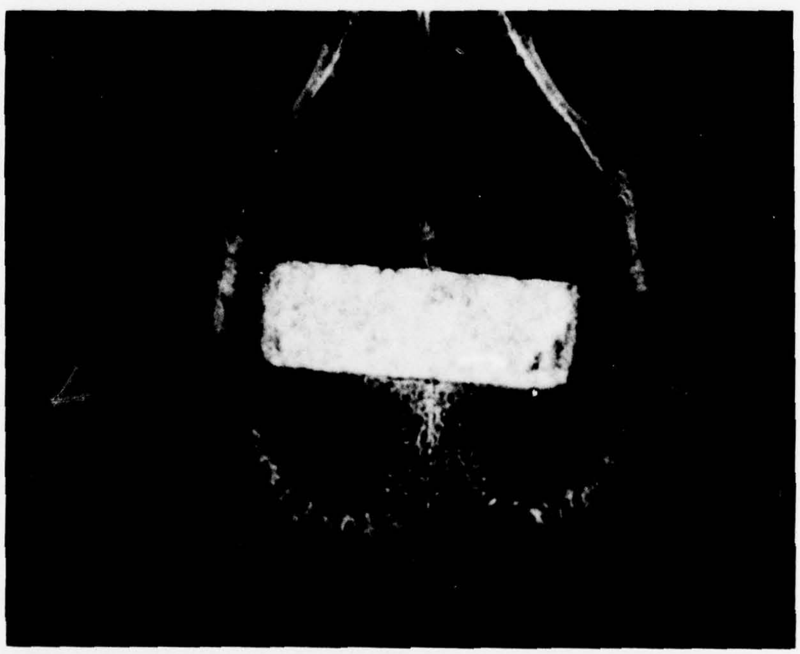


FIGURE 10. RADIOGRAPH OF EXCISED CALVARIUM FROM ANIMAL C 12 MONTHS POSTIMPLANT (HISTORIC STANDARD MATERIAL)

FIGURE 11. RADIOGRAPH OF ANIMAL K 3 MONTHS POSTIMPLANT (IMPROVED MATERIAL)

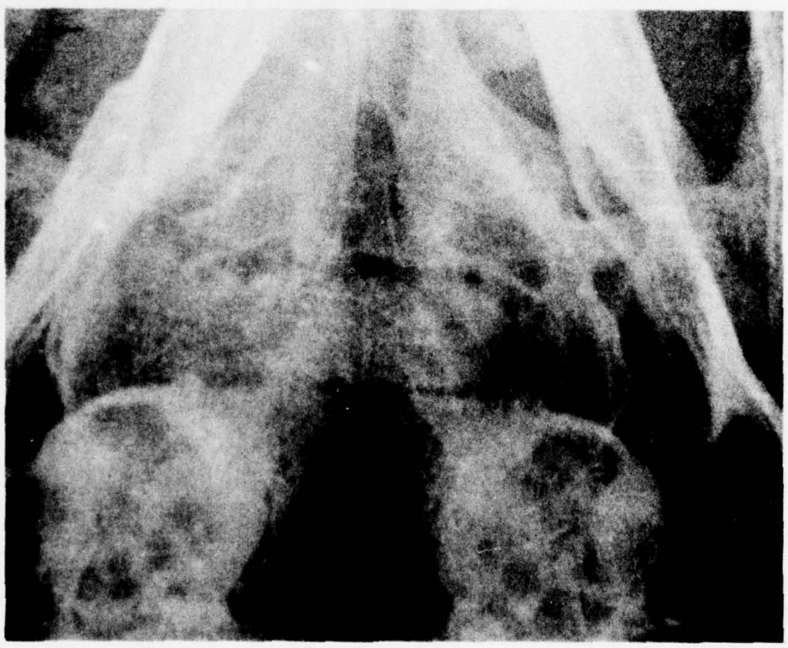


FIGURE 12. RADIOGRAPH OF ANIMAL K 6 MONTHS POSTIMPLANT (IMPROVED MATERIAL)

discern if it were not for the radiolucent boundaries. Figure 13 is Animal K at 9 months. In this radiograph, the implant is even more difficult to observe. Figure 14 shows Animal K at 12 months. Again, the implant proper is difficult to observe. Figure 15 shows a postnecropsy radiograph of Animal K with the calvarium excised. In this view, the radiodensity of the tricalcium phosphate appears to be similar to the surrounding bone; however, the area in which the implant was placed is still readily visible. Certain portions of the implant appear as natural bone would (invading across the interface) whereas other areas maintain the granular appearance of the implant.

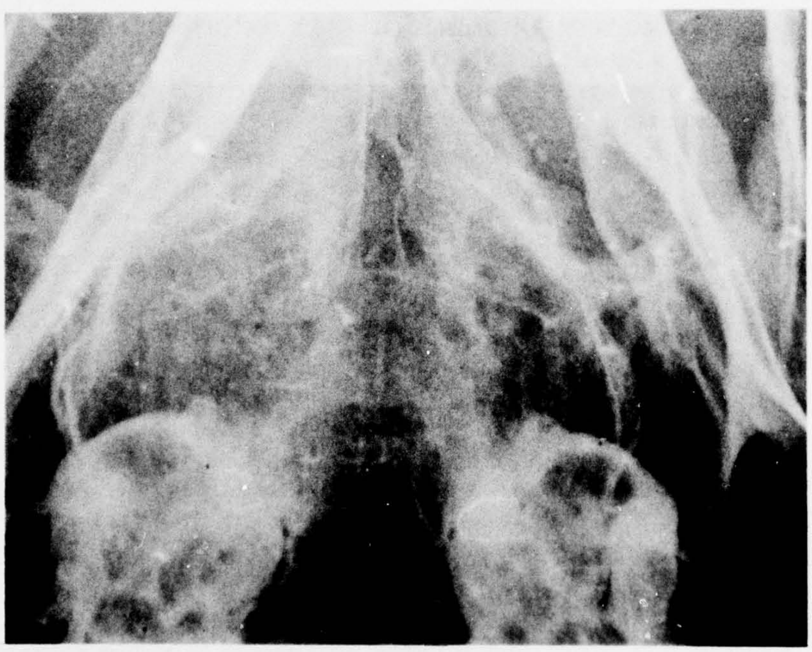


FIGURE 13. RADIOGRAPH OF ANIMAL K 9 MONTHS POSTIMPLANT (IMPROVED MATERIAL)

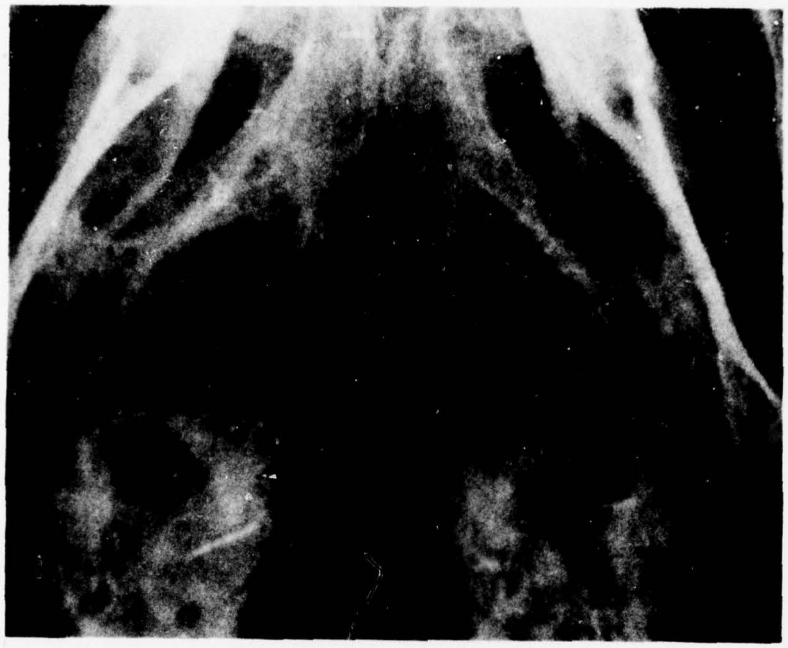


FIGURE 14. RADIOGRAPH OF ANIMAL K 12 MONTHS POSTIMPLANT (IMPROVED MATERIAL)

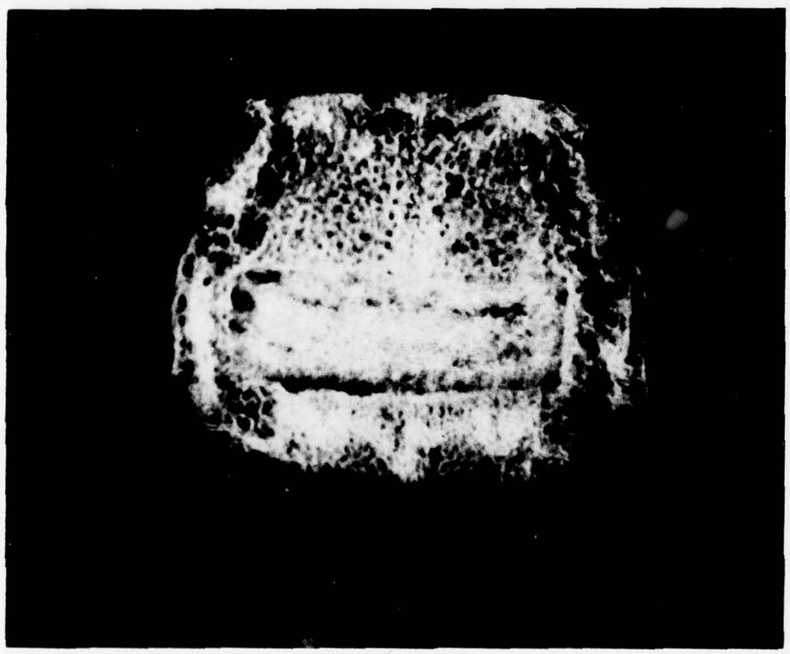


FIGURE 15. RADIOGRAPH OF EXCISED CALVARIUM FROM ANIMAL K 12 MONTHS POSTIMPLANT (IMPROVED MATERIAL)

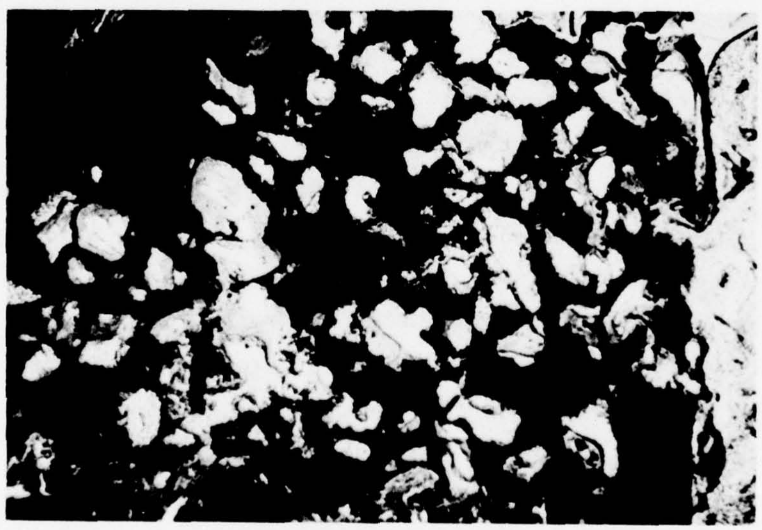
Histologic Evaluations

To evaluate the rate of ingrowth of the tricalcium phosphate, ground sections were prepared utilizing methyl methacrylate-embedded sections. Due to the nature of the tricalcium phosphate, sections cannot be prepared without embedding in a rigid fixation medium such as methyl methacrylate to maintain the integrity of the section. This is especially true with the shorter experimental ingrowth times. Slides have been prepared both prestained with fuchsin and also unstained. Histology in this report will all be basic fuchsin stained. The unstained sections are to allow for evaluation of the fluorescing dyes which have been injected at 3-month intervals prior to necropsy. Figure 16 is an example of the bone-tricalcium phosphate interface 3 months postimplant in the historic standard material. Note that good osseous formation can be seen intermingled within the irregular porosity of the tricalcium phosphate. However, connective tissue is also readily available. Figure 17 illustrates a similar view showing a greater depth into the implant proper. Notice the great irregularity in the tricalcium phosphate and as before the intermingling of connective tissue and bone. Connective tissue appeared somewhat more predominant in the larger porosities. Figure 18 is again the historic standard material 3 month postimplant at a higher magnification. Good osteoblastic activity is apparent well within the implant. It appears at this time that most available cavities throughout the tricalcium phosphate have been filled with a mixture of bone and connective tissue.

Figure 19 is the historic standard material 12 months postimplant. Note there is no great difference between this figure at 12 months and the earlier histology shown at 3 months. Apparently, biodegradation is not occurring rapidly. Since ingrowth is evident wherever tricalcium phosphate has degraded, degradation of the material appears to be the rate limiting step. Figure 20 illustrates at a higher magnification good osseous formation at the periosteal border of the implant after 12 months. This implant appears to be well surrounded by healthy bone formation.

10X

FIGURE 16. BONE-IMPLANT INTERFACE, HISTORIC STANDARD MATERIAL; ANIMAL A 3 MONTHS POSTIMPLANT; BASIC FUCHSIN

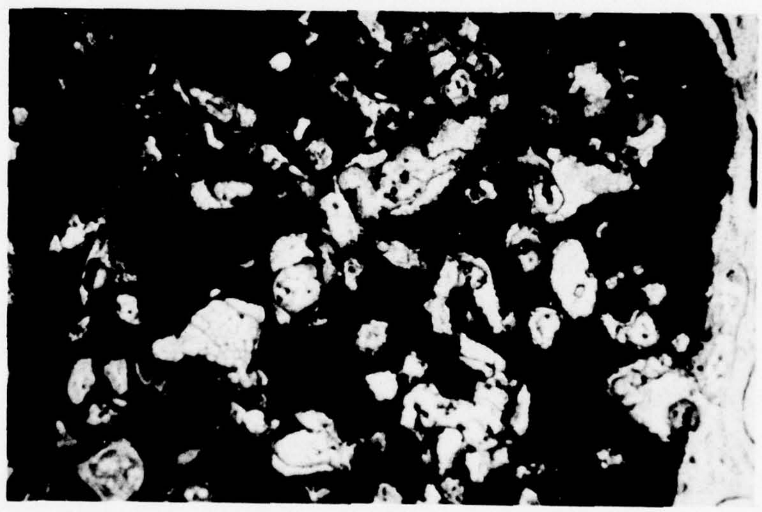


10X

FIGURE 17. HISTORIC STANDARD IMPLANT: ANIMAL A 3 MONTHS POSTIMPLANT

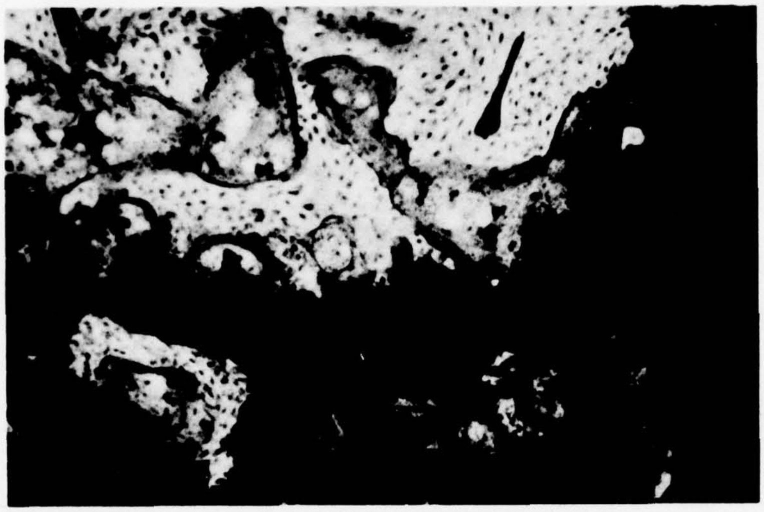
25X

FIGURE 18. CENTER OF IMPLANT, ANIMAL A 3 MONTHS POSTIMPLANT; HISTORIC STANDARD



10X

FIGURE 19. 12-MONTH IMPLANT, ANIMAL C; HISTORIC STANDARD



25X

FIGURE 20. 12-MONTH IMPLANT, ANIMAL C; PERIOSTEAL BORDER OVERGROWTH; HISTORIC STANDARD

In the improved tricalcium phosphate materials, a somewhat different pattern of biodegradability can be observed. Figure 21 illustrates an animal 3 months postimplant. Bone formation and/or connective tissue are seen in all of the pores, especially near the bone-implant interface. The pores closer to the implant interface contain more bone than those further away. However, some bone formation occurs in all pores throughout the material. Figure 22 shows the bone-implant interface at a higher magnification. The pores in this view are filled with established bone.

At 6 months (Figure 23), the situation is drastically different from that of the Historic Standard material. Note the good bony formation with some intermingling of connective tissue found throughout the view. The remaining tricalcium phosphate takes on an almost granular appearance, and in one corner appears almost powdered. However, the histology still appears normal. Figure 24 shows a 6-month implant at higher magnification. The granular appearance of the tricalcium phosphate is still readily

10X

FIGURE 21. NEW FORMULATION MATERIAL 3 MONTHS POSTIMPLANT, ANIMAL L

25X

FIGURE 22. NEW FORMULATION MATERIAL 3 MONTHS POSTIMPLANT, ANIMAL L

10X

FIGURE 23. IMPROVED MATERIAL 6 MONTHS POSTIMPLANT, ANIMAL E

63X

FIGURE 24, IMPROVED MATERIAL 6 MONTHS POSTIMPLANT; BONE FORMATION AREAS SURROUNDED BY GRANULAR TRICALCIUM PHOSPHATE; ANIMAL E

evident. This granular appearance is most predominant closer to the bone-implant interface. The osseous tissue appears normal within this area of implant.

Figure 25 shows the border region of an implant 9 months after surgery and Figure 26 shows a higher magnification view of the interior of the same implant. Here, the tricalcium phosphate does not appear quite as granular as the 6-month implant. However, the granular material at the interface is heavily invaded with connective tissue. At the higher magnification, the interior view shows good osseous formation well within the middle of the implant and very little granulation of the tricalcium phosphate as compared to Figure 24 .

At 12 months postimplant (Figure 27), this invasion of connective tissue into the remaining granularity is even more evident. However, notice that the bone formed apparently earlier is still intact and appears healthy. Figure 28 is the higher magnification view of this same implant. Note the bone formations surrounded by the connective tissue surrounding very small granules of tricalcium phosphate.

This change in the character of the tricalcium phosphate is interpreted to be due to a rapid loss of mechanical stability due to the accelerated biodegradability characteristics of the improved material. The rapid structure change is probably responsible for the large amount of connective tissue formation. It should be noted that at necropsy these samples were rigidly interlocked into the calvarium.

Blood and Urine Chemistry Profiles from Experimental Animals

Inorganic phosphorous determinations were made using an acid molybdate reaction. Calcium was determined with an ortho-cresolsphthalein complexone. Reagents for these tests were obtained from Dow Diagnostics. Blood was obtained from the rabbits for serum samples; 24-hour urine samples were collected from the rabbits by housing them in metabolic cages. The urine was well mixed and aliquots removed for 24-hour sample determination. Multiple determinations were made on all samples.

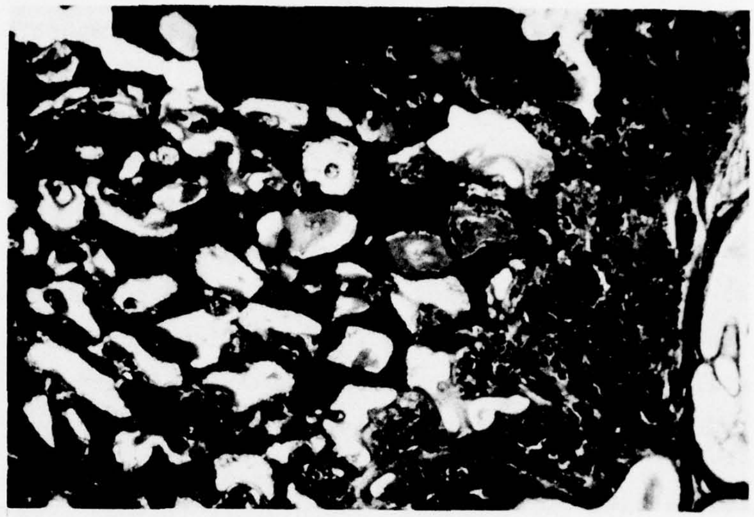


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FIGURE 25. IMPROVED MATERIAL 9 MONTHS POSTIMPLANT, ANIMAL F

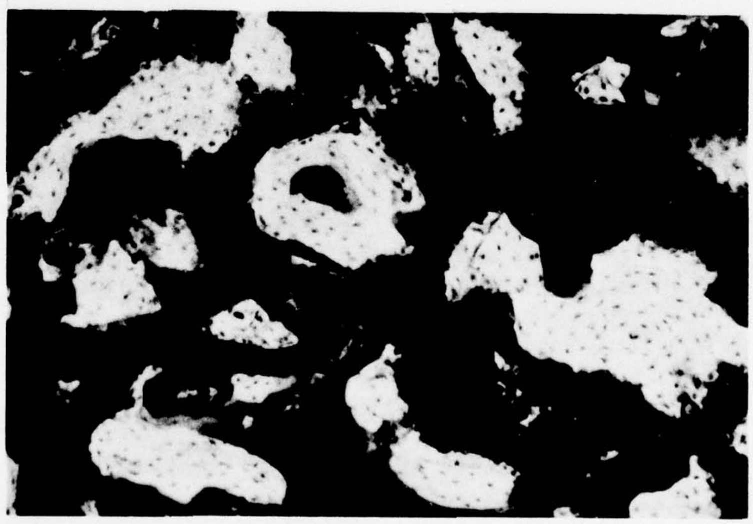

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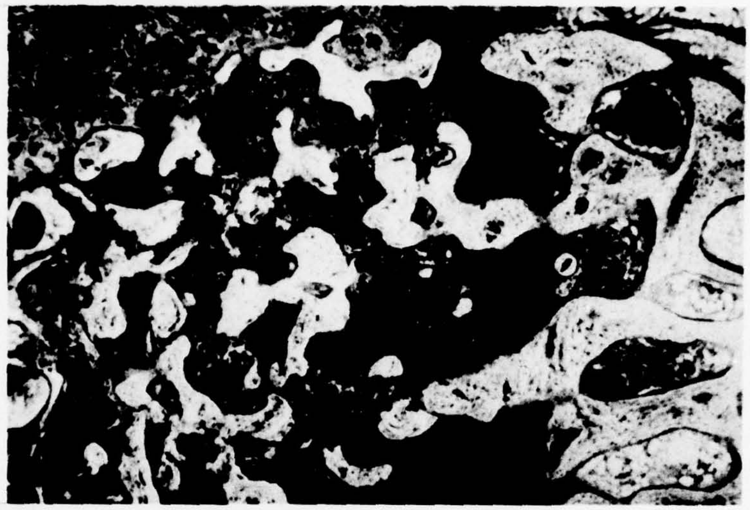
FIGURE 26. IMPROVED MATERIAL 9 MONTHS POSTIMPLANT, ANIMAL F



10X

FIGURE 27. IMPROVED MATERIAL 12 MONTHS POSTIMPLANT, ANIMAL K

25X

FIGURE 28. IMPROVED MATERIAL 12 MONTHS POSTIMPLANT, ANIMAL K

A large number of preoperative serum and urine samples were analyzed for controls. Control values for serum calcium averaged 7.39 mg/100 ml, S.D. = $\pm$ 0.3. Mean inorganic phosphorous values were 3.55 mg/100 ml, S.D. = $\pm$ 0.76. Mean urine calcium value was 11.7 mg/100 ml, S.D. = $\pm$ 5.78. Urine inorganic phosphorous mean value was 8.04 mg/100 ml, S.D. = 6.78. Figures 29-32 show one-year urine and blood values for both calcium and phosphorous. The results are graphed individually for Rabbits E through L. Rabbits E, F, G, and H indicate improved tricalcium phosphate implants in which the material was not radiolabeled with Ca^{45} during manufacture. Animals I, J, K, and L represent rabbits implanted with radiolabeled tricalcium phosphate. Notice in both Figures 29 and 30 there is very little shift of either serum calcium or serum inorganic phosphorous values throughout the 365 days of the experimental period. Only a few points marginally fall outside of $\pm$ 2 S.D. units range indicating significant shift. Figures 31 and 32 show graphically the urine chemistries for the unradiolabeled (Figure 31) and radiolabeled (Figure 32) animals. In both animal groups, there appeared to be some significant increases in the urine calcium, especially at the 6-month interval. However, this picture is not clearcut since fluctuations in urine chemistries are very irregular. The highly precipitous nature of urine makes obtaining samples difficult regardless of the analytical technique used to assess results.

Large and significant increases in urine inorganic phosphorous can be seen at several points throughout the experimental period. Most animals exhibited significantly higher levels at some point during the experiment. It is interesting to note that the animals do not exhibit continued elevated levels. Rabbits were reanalyzed wherever unusually large changes were noted. An analysis of results confirmed the data. The urine chemistries of these animals are probably indicative of the relatively rapid degradation of the tricalcium phosphate. It should be recalled that the animals illustrated in these graphs were implanted with new formulation tricalcium phosphate which biodegrades relatively rapidly.

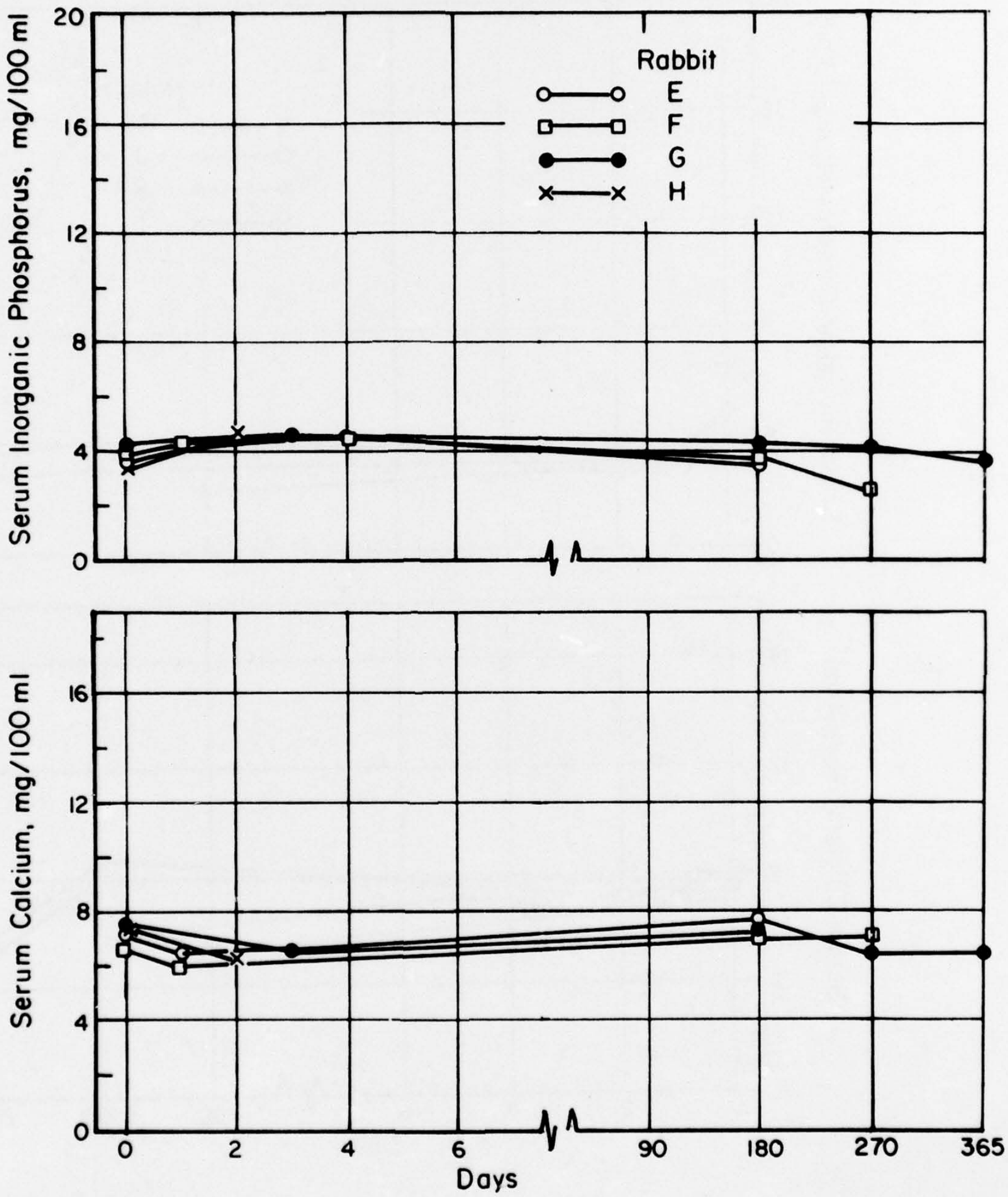


FIGURE 29. SERUM CALCIUM AND PHOPHOROUS LEVELS FOR THE IMPROVED TRICALCIUM PHOSPHATE IMPLANT ANIMALS E, F, G, AND H; NONRADIOLABELED MATERIAL (Day 0 = PREOPERATIVE)

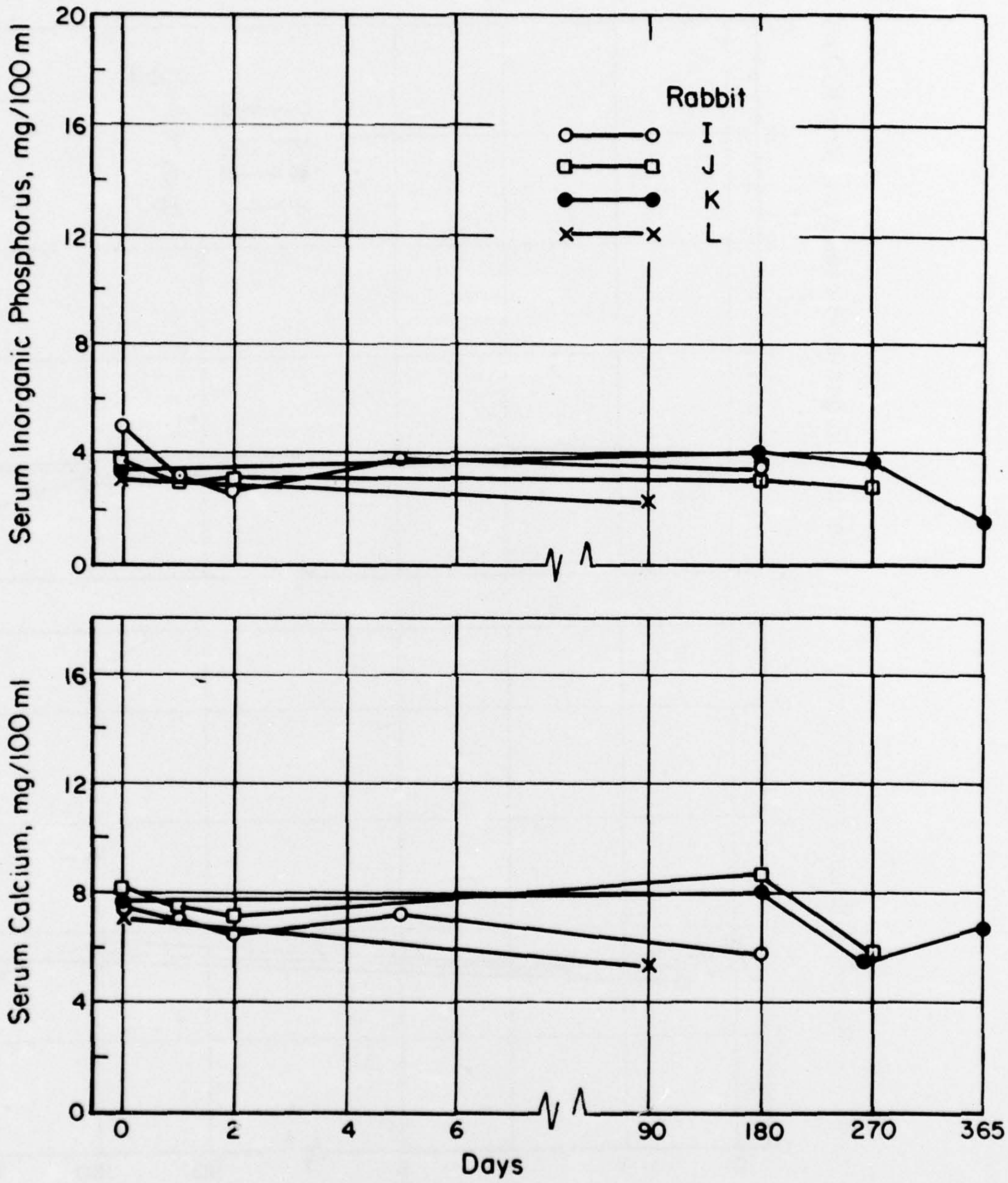


FIGURE 30. SERUM CALCIUM AND PHOSPHOROUS LEVELS FOR THE IMPROVED TRICALCIUM PHOSPHATE IMPLANT ANIMALS I, J, K, AND L; RADIOLABELED MATERIAL (DAY 0 = PREOPERATIVE)

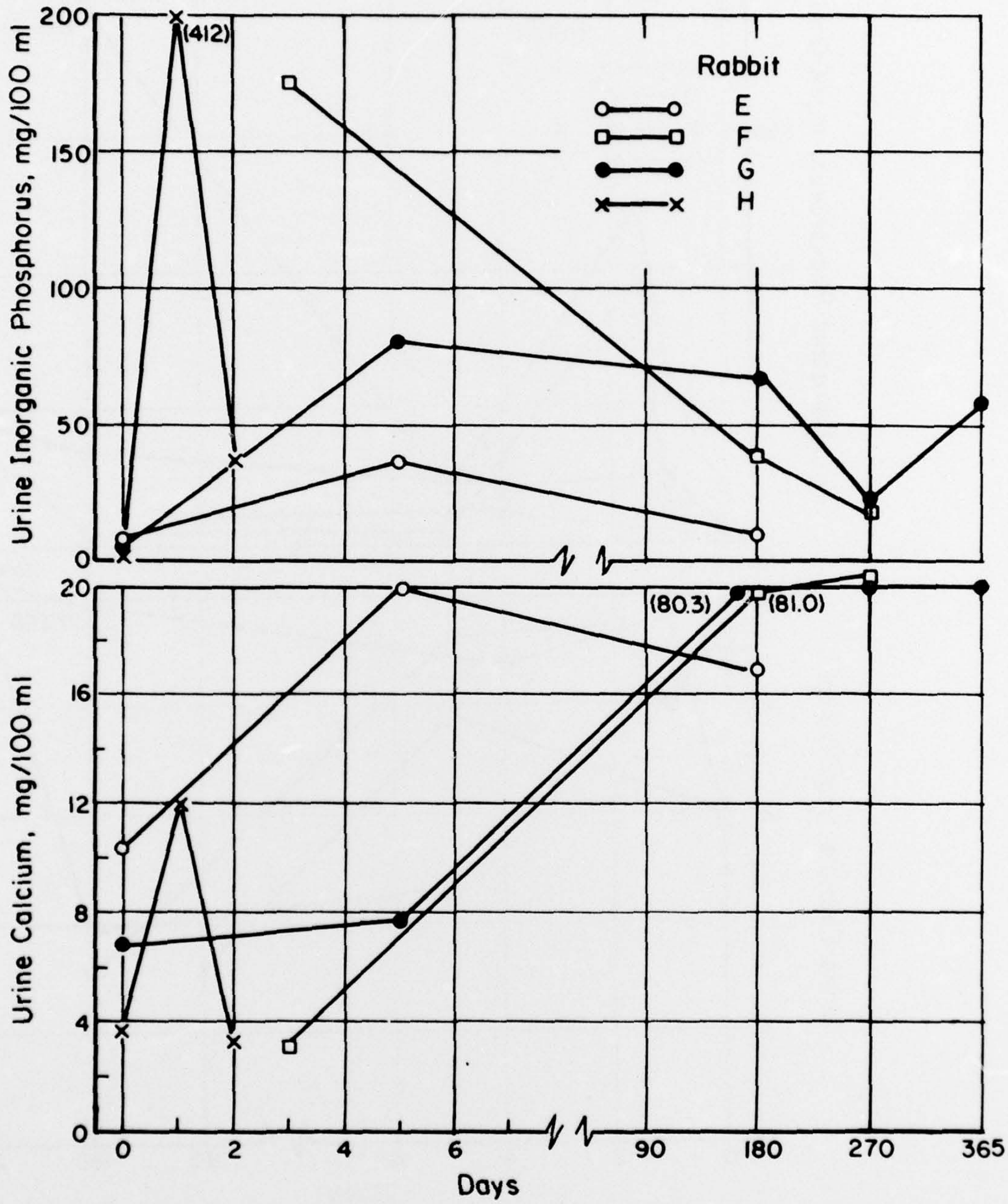


FIGURE 31. URINE CALCIUM AND PHOPHOROUS LEVELS FOR THE IMPROVED TRICALCIUM PHOSPHATE IMPLANT ANIMALS E, F, H, AND H; NONRADIOLABELED MATERIAL (Day 0 = PREOPERATIVE)

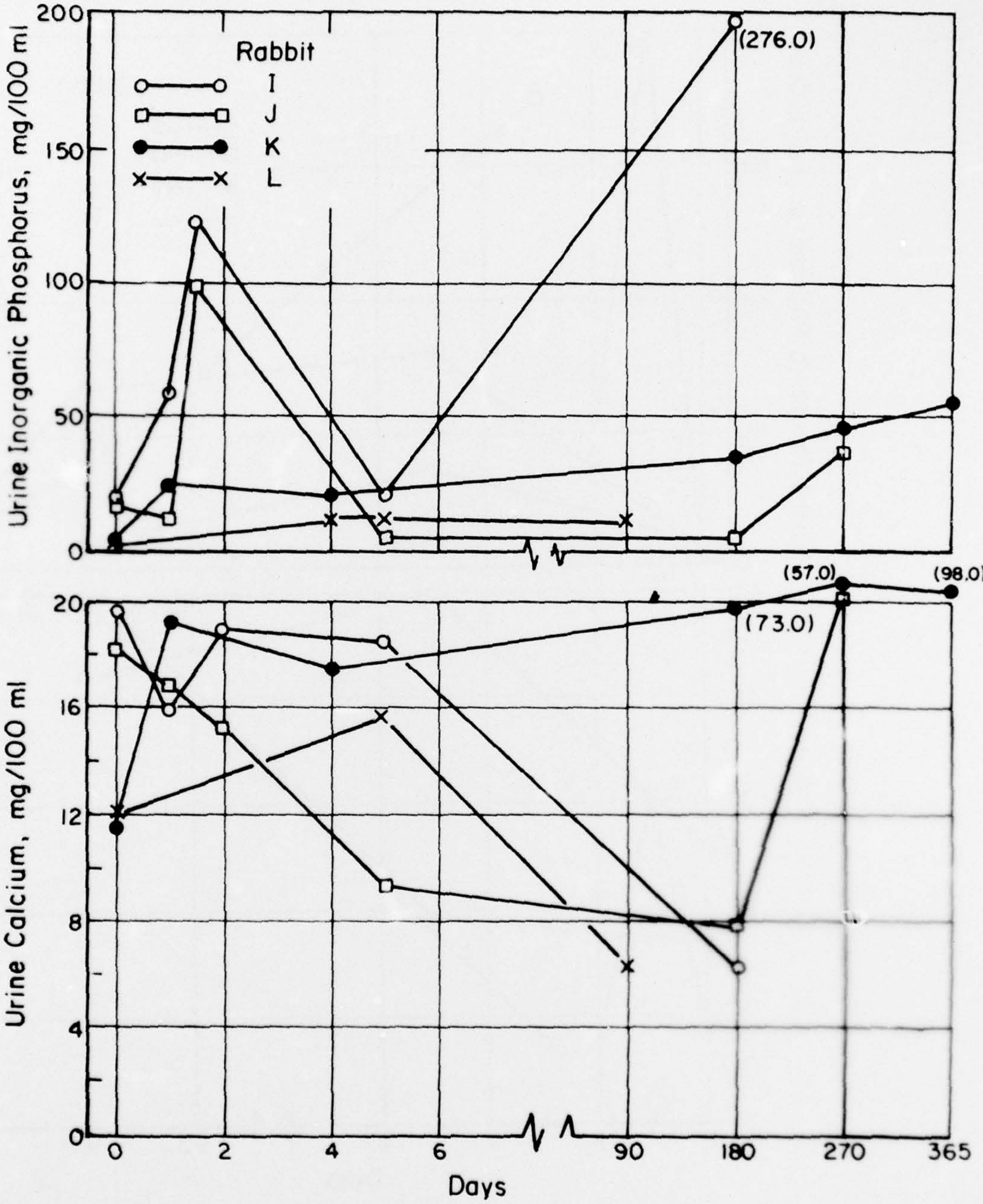


FIGURE 32. URINE CALCIUM AND PHOPHOROUS LEVELS FOR THE IMPROVED TRICALCIUM PHOSPHATE IMPLANT ANIMALS I, J, I, AND L; RADIOLABELED MATERIAL (DAY 0 = PREOPERATIVE)

Radioisotope Analysis of Tricalcium Phosphate Implant Animals

To assess the relative degree of biologic activity in and about the tricalcium phosphate implants as well as to ascertain the fate of calcium in the tricalcium phosphate radiolabeled implants, a series of radioisotope studies have been undertaken. Two radioisotopes are of interest in this study: Ca^{45} because of its importance in calcification and tritiated proline since proline is hydroxylated and incorporated in areas undergoing collagen synthesis and subsequent bone formation. Consequently, these two beta emitters serve as indices of bone metabolism and calcification. Additionally, the ultimate fate of tricalcium phosphate can be ascertained when radiolabeled Ca^{45} tricalcium phoshpate is implanted. Several scintillation-counting techniques were tried in the course of this research. Two major problems were found to exist. First, commercial solubilizers available for scintillation counting were found inadequate, and second, chemiluminescence obscured counts unless adequate precautions were taken. To overcome these problems, the method of Mahin and Lofberg [18] was modified for the digestion and counting of bone, tissue, urine, and plasma samples. Basically, the method consists of utilizing 0.2 cc fluid to be tested. A known mass of 40 mg or less is used in the case of solid specimens. To this is added 0.2 cc 60 percent perchloric acid followed by 0.4 cc 30 percent hydrogen peroxide. The sample is then heated in a 75 C oven for a minimum of 1 hour or until complete digestion of the sample occurs. The sample is cooled and neutralized with 2N sodium hydroxide. Fifteen ml of Instagel® is added to the scintillation vial. The vials are capped and placed in a dark refrigerator for a minimum of 24 hours. The samples are counted on either a Packard liquid scintillation counter or a Searle scintillation counter.

Figure 33 is an example of data expressed in CPM/mg obtained from one rabbit calvarium. The animal was injected with 400 μCi of tritiated proline and 200 μCi Ca^{45} 24 hours prior to necropsy. The data shown are for one-half of the calvarium. Using a low-speed diamond cutoff saw, the calvarium was severed at its midline and wafered into small rectangular cubes for counting purposes. The samples were weighed moist and then ground and crushed after being frozen in liquid nitrogen. The above-mentioned scintillation counting techniques were employed.

The raw data shown in this figure were corrected according to machine efficiency and decay and results tabulated in terms of disintegrations per minute per mg (DPM/min/mg). In an attempt to assess changes in overall biologic activity, average DPM/min/mg for the surrounding bone were calculated and compared to the average DPM/min/mg for the implant proper. The resulting implant-to-bone ratio is shown in Table 3 for both calcium and tritium. The use of ratios is demanded in this research since the data at each time period must, of necessity, be from different animals. The implant-to-bone ratios for both the tritium and Ca^{45} show an increase with time. This increase is indicative of increasing metabolic activity, i.e., collagen synthesis and calcification, within the implant. This is the expected result since with increasing biodegradability of the tricalcium phosphate, the volume of metabolizing bone would naturally increase, and consequently one would expect the ratio of biologic activity to eventually become the same as the surrounding material. Since implant material is always left in our samples at the end of 1 year, one would expect the numbers not to quite reach unity. However, the biologic activity seen in our samples is greater than unity in the 12-month specimens. This is probably caused by relatively high metabolic activity of newly forming bone in the implant. The amount of biologic data collected by this technique is still too small to attribute great significance to the technique. However, with time, confidence in techniques such as these is bound to improve. The method does assure that there is metabolic bone formation activity occurring within the sample.

RABBIT E - CPM/mg (H^3)

97	128	93.5	112	126	137
93.8	116	114	122	102	108
46	83.6	70.5	67.	65.4	61.
49.4	70.3	53.9	40.4	45.6	45
66.3	87.9	47.9	66.5	50.1	49.7
69.6	95.5	63.2	49	49.5	44.8
79.	64	62	50.9	39.7	64.6
43.4	91.1	89.1	64.2	61.5	

RABBIT E - CPM/mg (Ca^{45})

381.1	511.8	343.2	371.5	359.1	328.3
386.7	321.2	322.5	334.2	348.4	363.9
362.1	330.7	367.8	340.	343.6	352.4
305.7	337.1	290.1	242.6	271.5	279
328.3	397.7	257.6	294.9	340.8	332.6
316.7	377.6	310.7	258.9	277.5	278.1
360.2	379.5	366.3	318.8	322.3	363.3
324.7	347	366.2	336.9	294.2	

FIGURE 33. RADIOISOTOPE UPTAKE DATA FOR RABBIT E 6 MONTHS POSTIMPLANT

400 μCi H^3 proline and 200 μCi Ca^{45} were injected 24 hours prior to necropsy. The data are from one-half of the skull-implant area sectioned into cubes and individually counted. Data are expressed in counts per minute per milligram of sample. The cross-hatch area is the implant proper.

TABLE 3 . IMPLANT-TO-BONE AVERAGE UPTAKE RATIOS

Implant Time, mo.	H^3 Proline Bone	Aver. DPM/mg Implant	Ratio I/B
3	225	26.4	0.118
6	192	11.7	0.611
9	300.4	297.5	0.990
12	292.4	519.0	1.770

Implant Time, mo.	Ca^{45} Bone	Aver. DPM/mg Implant	Ratio I/B
3	1148.7	104.4	0.091
6	972.6	791.4	0.814
9	969.9	547.1	0.564
12	921.5	1366.9	1.480

Analysis of Radioisotope-Labeled Tricalcium Phosphate Implants

Implants with 50,000-100,000 DPM/mg of Ca^{45} incorporated in the tricalcium phosphate were implanted in the calvaria of 4 rabbits. These animals were subjected to all of the procedures listed for other animals in the study. The animals were run for periods of 3, 6, 9, and 12 months. Since there was Ca^{45} in the implant, Ca^{45} was not injected into the animal 24 hours prior to necropsy. Periodic urine, blood, and fecal samples were taken from these animals. At necropsy, biological material was taken from all major organ and tissue systems of the animal. Scintillation counting techniques similar to those previously described were utilized. With the exception of the implant proper and the immediate surrounding area, no statistically significant evidence of radioisotope activity could be found in any of the animals in any location. The only slight suggestion of radioisotope activity was in the long bones of animals which had been implanted in excess of 6 months. This result implies that there is no adverse accumulation of the calcium from the implant anywhere in the body. Presumably, then, the distribution of this material is in a normal fashion and is handled by the body no differently than on other calcium. The slow biodegradation of the material would not allow significant amounts of the material to be found except in body compartments where the calcium was relatively tightly bound. Slightly improved sensitivity of this experiment could be anticipated by increasing the total quantity of radioactivity in the original sample. However, adverse local effects occurring in the bone tissue surrounding the implant might be anticipated.

Conclusions and Recommendations

Material Processing Studies

The results of radiolabeled implant fabrication studies show that significant improvements in the chemical composition (Ca/P ratio) and structural uniformity of porous tricalcium phosphate materials can be achieved by the use of precise formulation and batching procedures in the preparation of the tricalcium phosphate powder coupled with the use of thorough and careful procedures for blending the phosphate powder with the naphthalene pore-forming agent. Compositions containing only 0.5 percent excess phosphorus (1.1 percent as P_2O_5) have been achieved. Further improvements may be possible with the use of iterative batching and analysis procedures.

Fabrication studies aimed at the preparation of porous tricalcium phosphate implant materials having controlled pore size distributions have revealed the need for further refinement of the process. The preparation of uniform and reproducible structures using reagent grade naphthalene as a fugitive pore-forming agent has been extremely difficult. Establishing valid cause and effect relationships has also been extremely difficult. The prime requirement is to achieve uniform and stable (non-segregating) phosphate/naphthalene powder blends. The blending process is sensitive to unknown variations between lots of reagent naphthalene, the freshness of the naphthalene, and the ambient conditions in the laboratory at the time of preparation. Significant process reproducibility and structural uniformity has been achieved by the use of technical grade naphthalene. Ultimately other processes for achieving porous ceramic structures such as foam techniques should be investigated.

Material Composition Studies

The results of studies initiated to determine the feasibility of developing porous $CaHPO_4$ as a new, potentially higher rate, bioresorbable implant material indicate that consolidated forms of $CaHPO_4$ can be prepared

by reactive hot-pressing techniques. Fine-grained specimens up to 70 percent dense have been prepared by this process. However, the preparation of specimens containing coarse porosity has not been possible because of adverse reactions with the pore forming agent (NaCl) during hot pressing. Further fabrication studies can not be recommended at this time. Comparative in vivo resorption rate studies should be conducted using the present 70 percent dense material in conjunction with 70 percent dense tricalcium phosphate as a standard. Should significantly favorable results be achieved, further fabrication studies might be warranted.

Biological Studies

The animal experiments show that tricalcium phosphate is indeed a biologically compatible biodegradable material. However, slight differences between formulations and structure of the material vastly alter its rate of biodegradability in the animal's system. Most notable is the difference between the phosphate-rich historical material and more nearly stoichiometric new formulation. The improved material with its accompanying increased mechanical integrity and more uniform pore structure biodegraded more rapidly, especially after the sixth month. However, starting in the vicinity of the ninth month, this new material degraded so rapidly that mechanical integrity of the implant appears to be altered and connective tissue invades about the remaining granular tricalcium phosphate. However, at necropsy, these samples are still rigidly interlocked in the bone and are not being sequestered. This result does not imply lack of biocompatibility in any fashion. However, it does mean that such a rapid degradation of material could be deleterious in stress-bearing situations. In comparison, the biodegradation of the older material was extremely slow. In fact, the rate of degradation is so slow that careful examination is necessary to ascertain much difference at all, especially in the period from the third through twelfth months of the experiment. Since the distribution of porosity as well as chemical nature of the material changed, the relative importance of these two factors must be considered together when drawing conclusions as to difference in degradability of these two materials.

These materials were not followed for longer than 12 months so it is difficult to assess the final result when both the implants completely disappear. Conceptually, longer term experiments should be run to assess the final result. These two materials show degradation extremes of a highly biocompatible implant material. The material could be tailor-made according to the results desired. For example, faster biodegradation on nonstress-bearing situations might be more useful in certain anatomical locations, whereas slower biodegradability exchanged for a more stable situation after the initial ingrowth might be more acceptable in locations where greater stresses are placed upon the system.

This research has indicated that a spectrum of evaluative techniques such as those employed in this report are necessary for a thorough understanding of the biodegradibility process. For example, one might unnecessarily condemn the newer formulation material if only histology were used for analysis. Of these techniques, histology and radiographic visualization are perhaps the most useful. However, it is expected with time that the other analysis procedures will become more meaningful when a greater data base is obtained.

Future studies should be directed toward determining independently the effects of variations in structure and composition on bioresorptivity.

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